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A system was designed and constructed to allow monitoring of blood flow by the use of Doppler flow probes. The Doppler signals are transmitted via an implanted FM transmitter to an external receiver, where the signal is processed and transformed to calibrated flow on a strip-chart recorder. This technique was used to measure the effect of inhaled or percutaneously administered nitroglycerin on coronary blood flow in dogs. The results of

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20 ABSTRACT (Continued)

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SUMMARY

A system was designed and constructed to allow monitoring of blood flow by the use of Doppler flow probes. The Doppler signals are transmitted via an implanted FM transmitter to an external receiver, where the signal is processed and transformed to calibrated flow on a strip-chart recorder. This technique was used to measure the effect of inhaled or percutaneously administered nitroglycerin on coronary blood flow in dogs. The results of the preliminary inhalation experiments indicate that the technique is valid for investigating whether nitroglycerin causes increased coronary flow and whether compensatory or reflex vasoconstriction occurs upon withdrawal. Both dogs exposed by inhalation seemed to demonstrate these phenomena slightly.

FOREWORD

In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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TABLES

INTRODUCTION

The toxicology and occupational health hazards of nitroglycerin, which has been used as a coronary vasodilator for many years, have been reviewed by Dacre and Rosenblatt (1974) and by Shiotsuka (1976) and will not be reiterated here.

Recently, concern has arisen that workers at ammunition plants may develop a tolerance to nitroglycerin while working and then suffer angina and/or sudden death upon withdrawal (termination, vacations) due to a postulated reflex coronary artery vasoconstriction mechanism.

Under Contract No. DAMD 17-76-C-6068, SRI International (formerly Stanford Research Institute) investigated the mechanism of tolerance and vasoconstriction during withdrawal using animal models. Specifically, we designed and developed implantable coronary flow probes with implantable telemetry devices. These were implanted in dogs, which were then exposed to nitroglycerin vapors or treated percutaneously with nitroglycerin for 10 days at a time.

Our preliminary findings demonstrate reduced coronary flow after 10 days of exposure to nitroglycerin. The preliminary data also suggest that some change in the biological half-life of the dinitroglycerins may occur after repeated daily exposures to nitroglycerin.

MATERIALS AND METHODS

Bioengineering

The bioengineering equipment was designed and assembled especially for these studies by personnel of the Stanford University Applied Electronics Laboratory. Figure 1 shows the basic system. It consists of implanted ultrasonic electronic flow probes, either an external (hard-wired) or an internal (implanted transmitter) telemetry link, and external Doppler processing electronics. The implanted electronics operate from a 2.8-volt implantable lithium battery, with a magnetic switch activated by an external magnet so that the system can be turned on only during data collection, thus conserving battery power.

For the flow probes, microcircuit techniques were used to assemble a small (2 mm x 2 mm) slab of piezoelectric ceramic into a biocompatible transducer assembly. The connections were made initially through small gold straps (later silver was used) between the transducer faces and the stainless-steel cable used to carry signals to either the external circuitry (in the hard-wired animals) or to the implanted telemetry package. The ceramic element is sandwiched between matching and backing layers of epoxy to provide efficiency and biocompatibility. The stainless-steel cables (as well as the implantable telemetry and power supply) were coated with Silastic after initial encapsulation in wax.

A wide-band FM telemetry link is used to transmit the Doppler shift information from the implanted electronics to an external receiver. Initially, this telemetry link was external to the dog, but later it also was implanted just beneath the skin. A two-transistor discrete transmitter is used (200 mHz medical telemetry band) with a range of greater than 3 meters. This band was chosen because of its normally low background noise. The signal is received on an FM telemetry receiver, which recovers the multiplexed Doppler information.

BLOCK DIAGRAM

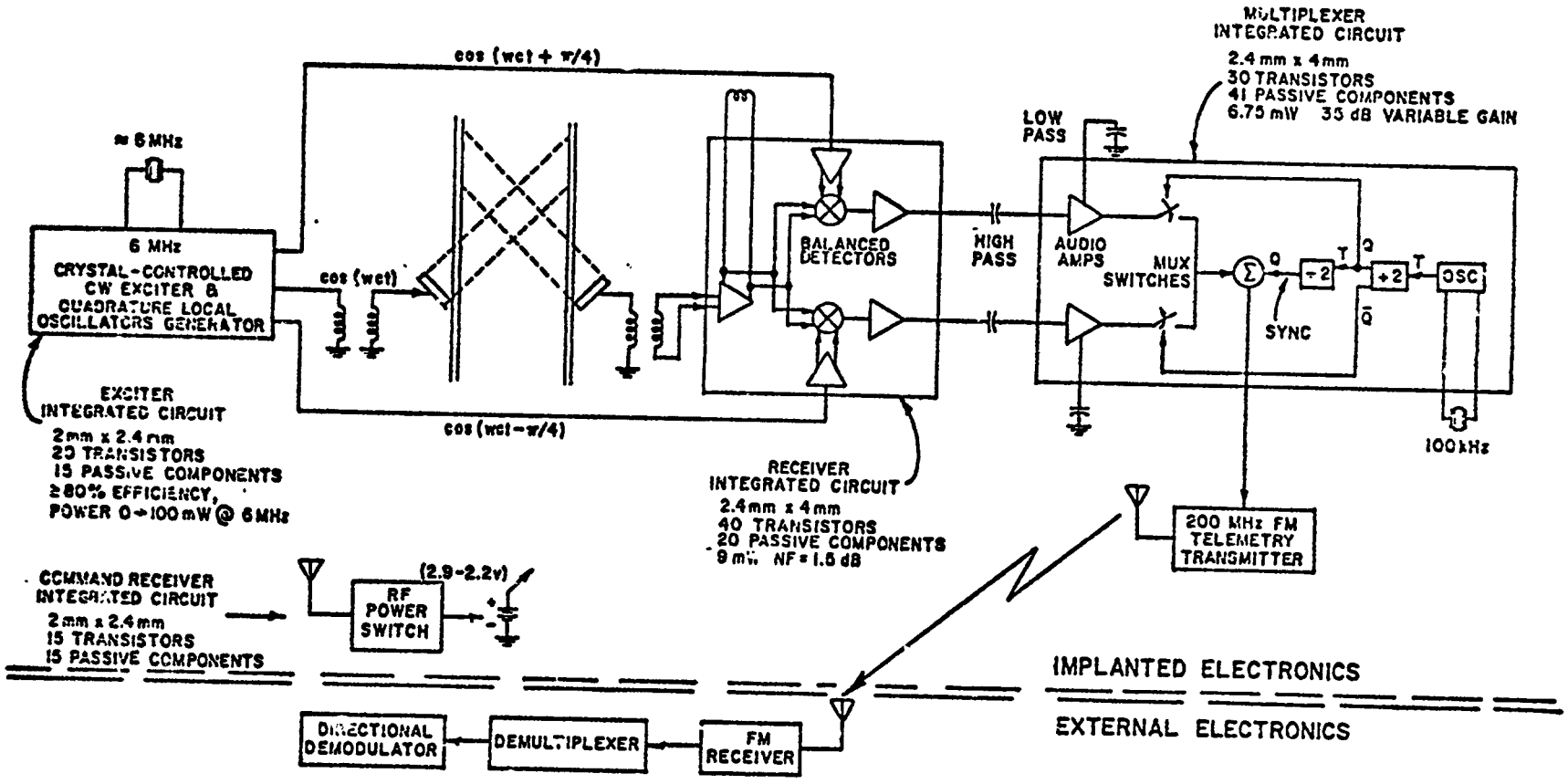

FIGURE 1 BLOCK DIAGRAM OF CW DOPPLER FLOWMETER

External signal processing electronics demultiplex the Doppler signals from the telemetry receiver. Further signal processing filters these signals and, through frequency to voltage conversion, convert them into a bidirectional flow signal for recording or display. A calibration of the system is shown in Figure 2.

Animals

The male and female beagles used for these studies were obtained from Marshall Farms, North Rose, New York. They were approximately 1 year ± 2 months of age on arrival. They were quarantined for a minimum of 3 weeks after their arrival at SRI. During that time, they were observed daily and examined by a veterinarian to ensure that only healthy dogs were used for the study. Dogs that had irregular heartbeats or respiratory patterns were eliminated from the study.

Surgery

All the dogs were bathed and then clipped free of hair over the thoracic area the day before surgery was scheduled. In addition, they were fasted overnight. Before surgery, they were anesthetized with sodium pentobarbital, 30 mg/kg intravenously. An intravenous drip of normal saline was established and maintained throughout the surgical procedure. Consequently, when administration of additional anesthetic or other agent was required, ready access was available through this route.

After anesthesia, the dog was thoroughly scrubbed over the area of incision with Phisohex, sprayed with tincture of Zepherin 1:1000, and draped with sterile linen. An incision was made on the left side, subcutaneous bleeders were clamped off, muscle layers were separated, and an incision was made through the sixth intercostal space. Just before its chest cavity was opened, the dog was placed on a Harvard respiratory pump and 100% oxygen was pumped into the respiratory system. The pericardium was opened and suspended around the intercostal incision with #4-0 silk suture to present the heart in a favorable aspect.

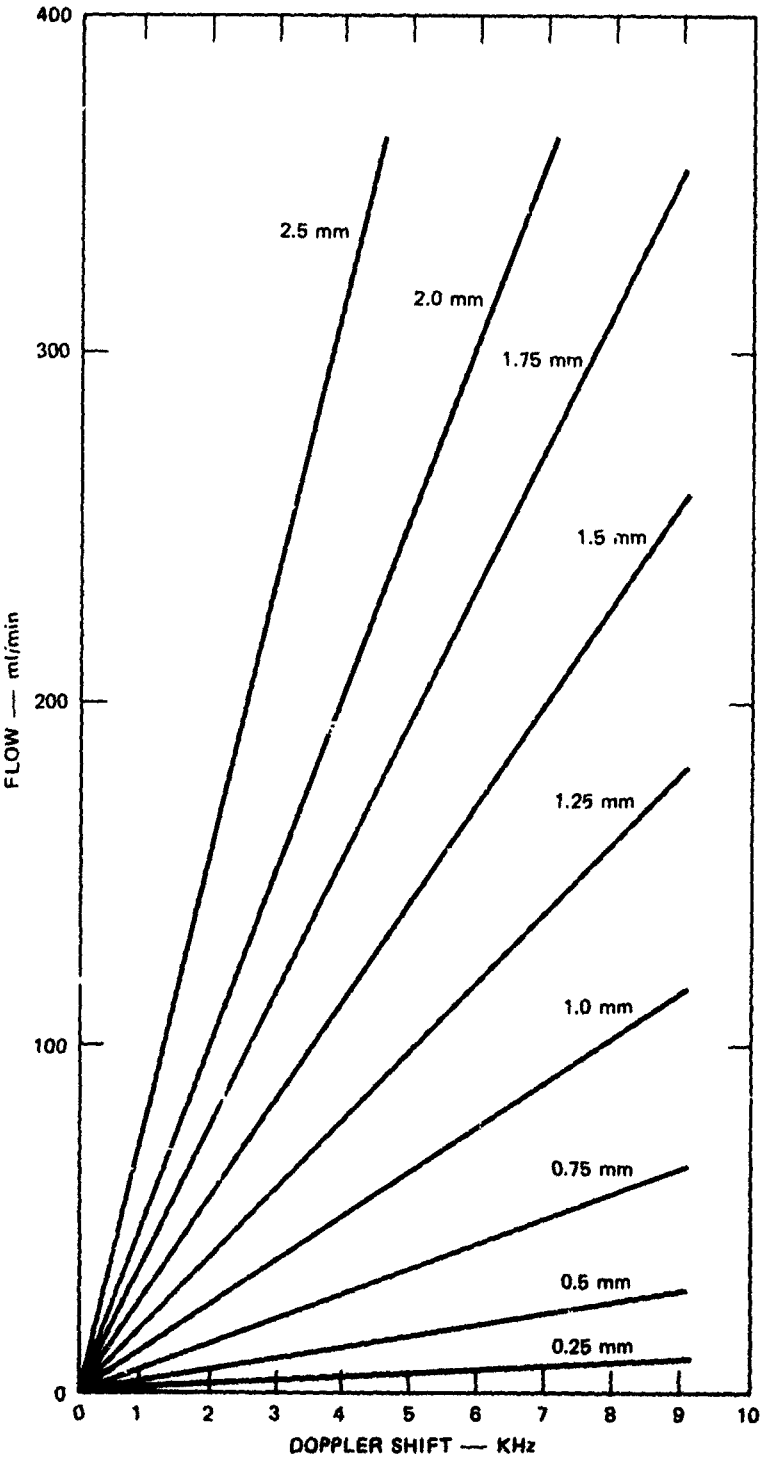

FIGURE 2 CALIBRATION FOR BLUNT PROFILE OR UNIFORM ILLUMINATION

1 KHz = CAL = 27 cc/sec $\Theta = 60^{\circ}$

Maximum Vessel Diameter = 2.5 mm.

Next, the left anterior descending coronary artery was visualized and teased away from its adjacent veins and fat pad by blunt dissection so that it was free from other tissue. The flow probe was then placed on the heart, and 4- to 5-mm-wide silastic strap was passed under the coronary artery to hold it up between the two Doppler flow probes without restricting flow in any way. Silastic flaps on top of the probe, as well as the strap running underneath the probes, were then secured to the myocardium with #4-0 silk suture and a 3/8 curve atraumatic needle.

The pericardium was closed carefully with #4-0 silk to avoid disturbing the imbedded vagus nerve, and the probe leads were brought out through the sixth intercostal space. The probe leads were secured to the rib with #1 silk so that normal pulling would not dislodge the flow probe from the coronary artery. The intercostal space was pulled together with double #1 silk suture, the muscle and fat layers were resected with 3-0 chromic, and the skin was closed with fine monel steel suture. If a telemetry package was also implanted, it was placed just beneath the skin. Later, we also suspended this from the rib sutures to prevent migration beneath the skin.

The animals were treated routinely with either penicillin-streptomycin or ampicillin for 5 days after surgery. Post-operative infection was not a problem in these studies, although some minimal evidence of rejection of the implants was observed in one case.

Generation of Nitroglycerin Vapors

Four glass cylinders, measuring approximately 10 cm by 30 cm, were filled with 800 g of loosely packed 10% nitroglycerin on lactose. Each end was stopped with a fiberglass wool packing and a #13 rubber stopper. A 0.5-inch glass tube in each rubber stopper provided an inlet and outlet for the generator. Four of these were connected in series so that breathing-quality air could be passed through them and then into the inhalation chamber.

The combined outlet from the four "generators" in series was directed into a 76 cm x 76 cm x 76 cm inhalation chamber. The chamber effluent

was directed out through a solution of sodium hydroxide-sodium hydrosulfide, which destroys nitroglycerin. The inhalation chamber, measuring $(76\ cm)^3$, was constructed of plywood and lined with a layer of fiberglass resin so that it could be destroyed by burning at the end of the project.

Percutaneous Exposures

For percutaneous administration, weighed quantities of 10% nitroglycerin on lactose were spread evenly on a 7.6 cm x 7.6 cm gauze pad. This pad was placed on the skin over the lumbar area of the dogs, which had previously been clipped free of hair. This was covered with a 15 cm x 15 cm sheet of heavy-duty aluminum foil. The gauze and aluminum foil were held in place with 5-cm roller gauze and tape.

Nitroglycerin Analysis

Atmospheric concentrations of trinitroglycerin were determined by gas chromatography (Varian Model 3740 gas chromatograph equipped with an electron capture detector). A 200 cm x 2 mm glass column was used that was packed with 3.5% QF-1 on Gas Chrom Q, 80/100 mesh. The column and injector temperature was 160° C and the detector temperature was 200° C. Nitrogen was the carrier gas with a flow of about 32 ml/min.

Preparation of Tritiated Nitroglycerin

Tritium-labeled nitroglycerin was prepared as follows. Labeled glycerin was obtained from New England Nuclear Corporation [2-^{3}H(N)] as a solution of 115 mg in 25 ml of sterile water. The water was removed under pressure before use in this synthesis.

A solution of 0.97 g (15.4 mmol) of 100% nitric acid in 10 ml of dichloromethane was added to a 50-ml round-bottom flask that contained 2.3 g (15.3 mmol) of trifluoromethanesulfonic acid. A viscous white solid appeared immediately at the bottom of the flask. A 103.5-mg (1.12-mmol) sample of tritiated glycerin dissolved in 0.4 g of anhydrous methanol was added to the above mixture, and the reaction was stirred for 2.5 hr at room temperature. The resulting two-phase mixture was

allowed to stand for 15 min. The upper phase was separated and the lower phase was washed two times with 20 drops of dichloromethane, which was then combined with the upper phase. The combined liquid was passed through a silica gel and "Woelm" aluminum oxide (basic activity grade 1) column. The evaporation of one-tenth weight of the filtrate under vacuum yielded 10.2 mg of a viscous, light, green-yellow liquid. The infrared spectrum was identical with that of an authentic sample of nitroglycerin. The total yield based on this aliquot was 40%.

Blood Levels of Nitroglycerin

Blood levels of tritiated nitroglycerins were determined by separation of the mono-, di-, and tri-nitroglycerin on silica gel plates and elution and quantitation using a liquid scintillation spectrometer.

Precoated silica gel 60 plates (with fluorescent indicator, 0.25 mm thickness, E. M. Laboratories, Inc., Elmsford, New York) were used for all experiments. Samples were spotted 2.0 cm and developed for a minimum of 10 cm. The solvent used was benzene:ethyl acetate (4:1).

Blood samples were drawn into a solution of mercuric chloride to stop all enzymatic activity of serum and red cells from further degradation of nitroglycerins. The samples were extracted into ether and then spotted on silica gel plates. Ether extracts the dinitroglycerins and trinitroglycerins quantitatively but only extracts the mononitroglycerins to the extent of 65%. Therefore, a correction factor was used to calculate the concentrations of mononitroglycerins.

Pathology

Tissue sections of the coronary artery and the adjacent myocardium were taken from dogs when the probes were no longer functional. The tissues were fixed routinely in 10% neutral buffered formalin and stained with eosin and hematoxylin.

RESULTS

Seven hard-wired coronary flow probes and seven flow probes with implanted telemetry packages were implanted in beagles. Table 1 lists the type of implant each dog received. Although many problems were encountered (see Appendix), some data were obtained.

A beagle was injected intravenously with 1.0 mg of nitroglycerin in 50% ethanol (10 mg of nitroglycerin per milliliter of solution). An immediate increase in mean coronary flow was observed with slight increase in the magnitude of the pulsatile flow. A similar volume of 50% ethanol produced just the opposite effect. After its coronary flow returned to baseline, the dog was again injected with 2.5 mg of trinitroglycerin. Figure 3 shows the effects of this injection. A prompt increase in the magnitude of the pulsatile coronary flow occurred and the mean flow rate nearly doubled. Therefore, this system apparently can detect changes in coronary flow produced by intravenous nitroglycerin.

Two dogs were exposed for 1 hr in the inhalation chamber to nitroglycerin vapors. During the exposures, the magnitude of the pulsatile flow increased as the exposure progressed, indicating that some effect was obtained from the inhaled nitroglycerin vapors. However, we had some difficulty in obtaining adequate chamber samples for concentration analysis.

Dog NG-8 was exposed daily for 6 hr to nitroglycerin vapors for 10 days. The vapor concentrations in the chamber, shown in Table 2, were extremely low and variable while the desired concentrations were going into the chamber (~5 mg/m^3). The dog apparently absorbed a great amount of the nitroglycerin on its fur, making prediction of inhaled doses nearly impossible. Table 3 summarizes the heart rate and mean coronary flow data collected before, during, and immediately after exposure each day in dog NG-8. This dog was doing well until the sixth

Table 1

DOGS IMPLANTED WITH CORONARY FLOW PROBES
AND PROBES WITH TELEMETRY PACKAGES

Dog No.	Implant	Remarks
NG-1	Hard wire	Probe not functioning after 60 days
NG-2	Hard wire	Chewed off leads
NG-3	Hard wire	Chewed off leads
NG-4	Hard wire	Chewed off leads
NG-5	Telemetry package	Died of unknown causes
NG-6	Telemetry package	Acute studies
NG-7	Telemetry package	Acute studies
NG-8	Telemetry package	Used for 10-day inhalation study
NG-9	Hard wire	Acute studies; chewed off leads
NG-13	Telemetry package	Transmitter failure (dead battery?)
NG-15	Hard wire	10-Day dermal study
NG-17	Telemetry package Battery replaced	10-Day inhalation study 10-Day dermal study (in progress)
NG-19	Hard wire	Probe failure
NG-21	Telemetry package	10-Day dermal study (in progress)

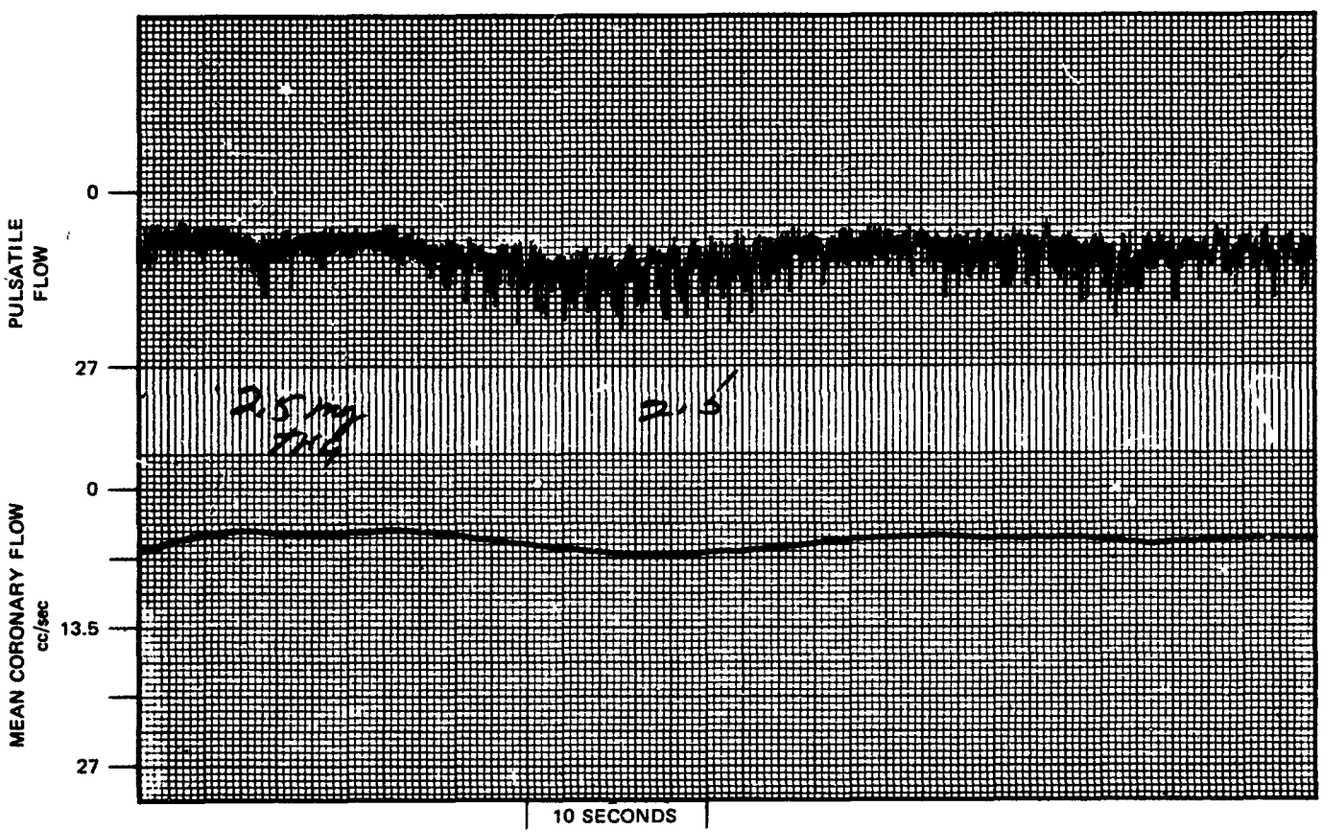

FIGURE 3 PULSATILE FLOW AND MEAN CORONARY FLOW IN A DOG AFTER THE INTRAVENOUS INJECTION OF 2.5 mg OF TRINITROGLYCERIN

Table 2

DAILY CHAMBER CONCENTRATION OF NITROGLYCERIN
DURING EXPOSURE OF DOG NG-8
(mg/m^3)

Day	Mean Concentration and Range	
	Inlet	Chamber
1	--	0.202 (0.10-0.21)
2	--	0.151 (0.01-0.43)
3	--	0.29 (0.02-1.44)
4	--	0.227 (0.01-1.4)
5	--	0.283 (0.01-2.32)
6	5.6	0.31 (0.02-1.91)
7	3.37	0.02
8	--	0.314 (0.06-0.54)
9	--	0.324 (0.05-1.70)
10	--	0.221 (0.04-0.25)

Table 3

HEART RATE AND CORONARY FLOW IN DOG NG-8
DURING EXPOSURE TO VAPORS OF TRINITROGLYCERIN FOR 6 HOURS/DAY

Day	Heart Rate	Coronary Flow (cc/sec)	Remarks
1	102	7.1	Baseline
	90	5.1	Resting in chamber
	135	7.7	TNG off
2	88	7.1	In chamber
	108	8.4	TNG on
	68	6.4	Resting
	68	6.4	Resting
	75	8.4	Walking
	180	5.8 - 8.4	TNG off
	138	7.7 - 8.4	Out of chamber
3	144	6.4	Walking on floor
	88	5.8	In chamber
	104	6.4 - 7.1	TNG on
	60	5.1 - 6.4	Resting in chamber
	126	6.4 - 7.1	Chamber open
4	124	7.1 - 7.7	Walking
	84	6.4 - 7.1	Resting in chamber
	112	6.4 - 7.1	Chamber open
5	92	6.4	TNC on
	88	6.4	Resting
	100	7.1 - 7.7	TNG off
6	100	8.4	TNG on
	87	7.1 - 7.7	TNG off
7	Unusable		
8	120	4.6 - 5.3	Walking
9	--	4.0 - 4.6	In chamber
10	Unusable		

day. when we were unable to activate the magnetic switch to turn on the transmitter and collect data during the exposure. On the seventh day we were unable to activate the switch at all, and on the eighth and ninth days we were able to keep it on only once. This was most unfortunate because some reduced flow was indicated on the eighth and ninth exposure days. We were unable to collect any data during the recovery period. Figure 4 shows the increase in pulsatile and mean flow observed daily as the nitroglycerin exposures began. This was observed in both dogs given an inhalation exposure.

A second dog, NG-17, was started on the daily exposure regimen (6 hr/day for 10 days). Table 4 shows the chamber concentrations. Again, the inlet concentrations were in the desired range but the chamber concentrations were very low. The chamber concentrations increased daily, as if the dog's fur were becoming saturated. Table 5 presents the daily heart rate and coronary flow data. Again, we had some difficulty in manipulating the magnetic switch. In general, the coronary flow seemed to be most improved (increased) from the sixth day of exposure. However, this may reflect an increase in chamber concentration during the last 6 days. On the eleventh day (24 hr after the last exposure), the coronary flow was reduced below those levels obtained at that heart rate. In fact, the coronary flow appeared to be beginning to slow during the last 2 exposure days.

Dog NG-15 was placed on a percutaneous treatment regimen (6 hr/day for 10 days). It was treated with 2.0 g of 10% nitroglycerin on lactose or 200 mg of nitroglycerin under an occluded percutaneous bandage. Table 6 presents the heart rate and coronary flow data. Generally, the coronary flow increased slightly or remained unchanged during each daily treatment. The overall trend was a slight general decrease in coronary flow. Two days after the treatment period ended, the flow was at its lowest for that heart rate.

The plasma levels of tritiated nitroglycerin were examined in dogs to determine whether nitroglycerin is handled differently after 10 daily exposures to its vapors. In an initial experiment, we determined

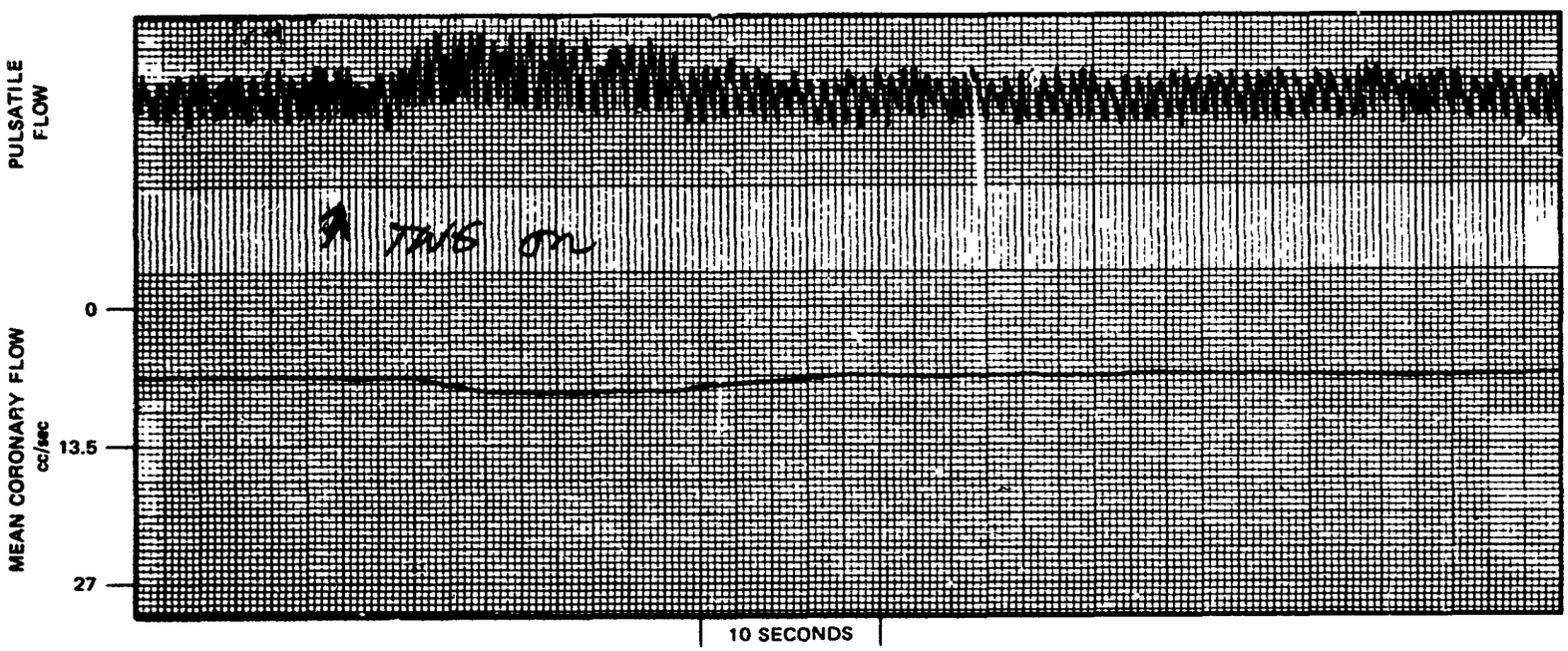

FIGURE 4 TYPICAL CHANGES IN PULSATILE FLOW AND MEAN CORONARY FLOW WHEN THE NITROGLYCERIN VAPOR GENERATORS WERE TURNED ON EACH MORNING (Dog NG-8)

Table 4

DAILY CHAMBER CONCENTRATIONS OF NITROGLYCERIN
DURING EXPOSURE OF DOG NG-17
(mg/m^3)

Day	Mean Concentration and Range	
	Inlet	Chamber
1	--	0.09 (0.01-0.27)
2	--	0.068 (0.01-0.30)
3	--	0.037 (0.01-0.08)
4	--	0.01 (0.002-0.03)
5	6.35	0.46 (0.12-0.85)
6	--	0.433 (0.19-0.96)
7	7.41	0.545 (0.30-0.76)
8	7.50	0.8
9	11.54	1.33 (1.06-1.70)
10	2.30	1.67 (0.06-3.91)

Table 5

HEART RATE AND CORONARY FLOW IN DOG NG-17
DURING EXPOSURES TO VAPORS OF TRINITROGLYCERIN FOR 6 HOURS/DAY

Day	Heart Rate	Coronary Flow (cc/sec)	Remarks
1	140	3.9 - 4.5	TNG on
	140	3.2 - 3.9	Out of chamber
2	144	4.0	
	124	2.3	
	116	3.0	
3	140	3.4	
	104	2.7	
	88	1.7 - 2.0	
	92	2.4	
	128	3.0 - 3.4	
4	160	4.6	
	96	3.3	
	88	3.3	
	184	2.6	
5	156	3.9	
	168	--	
6	156	5.3 - 6.6	
	96	4.6	
	136	5.9 - 7.3	
7	132	5.9	In chamber
	160	6.6	TNG or
	128	4.6	
	108	5.9	
	96	5.9 - 7.2	
	120	5.9	
	104	4.6	
	180	7.9 - 8.6	Out of chamber
	164	7.2	10 min $\bar{p}$, out of chamber
	152	5.9	20 min $\bar{p}$, out of chamber
	136	5.9	30 min $\bar{p}$, out of chamber

(Continued)

Table 5 (Concluded)

Day	Heart Rate	Coronary Flow (cc/sec)	Remarks
8	144	4.7	
	164	6.8	
	136	4.1 - 5.4	
	128	4.1 - 4.7	
	116	4.1	
9	148	5.4	
	152	4.7 - 5.4	
	132	4.7 - 5.4	
10	128	4.7	
	152	4.1	
	140	3.3 - 4.1	
11	120	2.7	

Table 6

HEART RATE AND CORONARY FLOW IN DOG NG-15
DURING DERMAL EXPOSURES TO TRINITROGLYCERIN FOR 6 HOURS/DAY

Day	Heart Rate	Coronary Flow (cc/sec)	Remarks
1	156	4.0	Standing
	128	4.7 - 5.4	
	140	4.7	
2	132	3.3	
	136	4.0	
3	144	4.0	
	140	4.0	
	204	4.0	
	144	3.3 - 4.0	
4	152	4.0	
	144	4.7 -	
5	136	3.3	
	132	3.3	
	136	3.3	
6	136	2.0	
	192	4.0	
	156	2.0 - 2.6	
7	160	2.0	
	128	2.0	
	156	2.0 - 2.6	
	144	1.4	
8	1/2	3.3 - 4.0	
	148	3.3	
	152	3.3	
	160	3.3 - 4.0	

(Continued)

Table 6 (Concluded)

Day	Heart Rate	Coronary Flow (cc/sec)	Remarks
9	160	4.0	
	148	2.4 - 2.6	
	152	2.0 - 2.6	
	160	2.0 - 2.6	
10	168	3.4	Last day of percutaneous treatment
	168	2.6 - 3.4	
	144	2.0 - 2.6	
	164	3.4	
	160	2.7	
11			
12	136	2.0	

the optimal time to sample for blood levels of the various nitroglycerins. The results of this study, shown in Table 7, indicated that most of the tri- and di-nitroglycerins were degraded in less than 1 hr. Therefore, in the following studies samples were taken sooner and more frequently.

Dog NG-8 was treated before and after the 10-day exposure with tritiated nitroglycerin. The results, presented in Table 8, indicate that after the 10-day treatment the biological half-time was somewhat longer compared with the pre-exposure half-time. This is interesting because the half-time would be expected to be decreased, if anything, by enzyme induction or a similar mechanism. Because the post-inhalation assay was done 24 hr after the last inhalation exposure, no resident nitroglycerin would have been left to hinder the metabolic disposition of the tritiated nitroglycerin. Table 9 shows the pre-exposure data from four other dogs that had been treated either by inhalation (NG-17) or by percutaneous administration of nitroglycerin. The data show that trinitroglycerin is rapidly converted to the dinitroglycerins in vivo. The half-time of 1,3-dinitroglycerin ranges from 25 to 50 min, and the half-time of 1,2-dinitroglycerin ranges from 22 to 29 min. The mononitroglycerins are more stable.

Dogs that had electronic and/or probe failures were autopsied, and the hardware was removed for recycling and repair. Tissues were taken from beneath and near the probe implant site on the heart. The sections included the coronary artery and the adjacent myocardium. In dogs NG-8 and NG-13 (the only two in which the histopathologic examination has been completed), the coronary arteries were not affected by the implantation of the coronary flow probe. A mild fibrosic and capillary proliferation was apparent around the coronary artery, but it did not involve the artery. No inflamation was evident. Therefore, the coronary flow probe did not cause any problem that would preclude its use in such a study.

Table 7

DISTRIBUTION OF RADIOACTIVITY IN BLOOD FROM DOGS AFTER INJECTION WITH 0.5 mCi OF TRITIATED NITROGLYCERIN

	Percentage of Radioactivity at Time (hours):			
	1	2	3	4
TNG	11.3	6.5	3.9	6.1
1,3-DNT	5.1	3.9	7.7	2.3
1,2-DNG	23.7	11.6	21.0	4.4
MNG	43.6	66.9	53.9	72.4
Origin	16.2	11.1	13.5	14.8

Table 8

DISTRIBUTION OF COUNTS FROM INJECTED TRITIATED NITROGLYCERIN
BEFORE AND AFTER 10 DAYS OF INHALATION
OF NITROGLYCERIN VAPORS IN DOG NG-8

	Time (min) After ^{3}H-TNG Injection					
	10	20	30	60	120	$T_{\frac{1}{2}}$
Before 10-day exposure						
TNG	0.2	--	0.4	0.4	0.2	
1,3-DNG	1.4	--	1.0	0.6	0.3	49.8
1,2-DNG	12.2	--	6.0	1.4	0.6	25.3
MNG	11.3	--	16.2	17.8	11.1	
After 10-day exposure						
TNG	1.5	0.8	1.1	0.7	1.4	
1,3-DNG	3.6	4.9	4.1	1.8	1.5	66.3
1,2-DNG	29.8	22.2	17.4	9.2	3.3	35.3
MNG	8.3	15.8	18.1	22.9	17.9	

Table 9

DISTRIBUTION OF COUNTS FROM INJECTED TRITIATED NITROGLYCERIN IN DOGS NG-13, NG-15, NG-17, AND NG-19

	Time (min) After ^{3}H-TNG Injection					
	10	20	30	60	120	$T_{\frac{1}{2}}$
NG-13						
TNG	0.3	--	0.3	0.7	0.2	
1,3-DNG	2.5	--	1.1	0.6	0.2	31.7
1,2-DNG	8.6	--	5.2	1.8	0.3	22.4
MNG	10.2	--	11.5	11.7	8.1	
NG-15						
TNG	0.3	0.3	0.2	0.2	0.1	
1,3-DNG	5.7	4.8	2.8	2.1	0.5	32.3
1,2-DNG	7.2	7.9	7.4	2.4	0.7	29.7
MNG	15.0	13.2	14.3	15.8	10.9	
NG-17						
TNG	0.2	0.2	0.2	0.2	0.1	24.2
1,3-DNG	4.2	4.3	1.9	0.8	0.2	34.4
1,2-DNG	8.8	6.3	5.8	1.8	1.0	
MNG	14.3	12.4	14.3	12.4	9.3	
NG-19						
TNG	0.8	0.3	0.2	0.4	0.8	
1,3-DNG	4.9	1.7	3.2	1.1	0.7	45.2
1,2-DNG	10.2	5.6	3.8	2.1	0.6	29.0
MNG	13.2	15.6	16.6	15.0	9.7	

DISCUSSION

Inhalation exposures to nitroglycerin are difficult to assess in terms of the total dose being given because of the wide variation between the concentration going into the chamber, the concentration of samples from the chamber itself, or the chamber exhaust. All the analytical samples were analyzed by the same gas chromatographic technique so the differences detected are most likely not an analytical error. Probably the nitroglycerin absorbs on the dog's fur and possibly on the chamber walls as well.

Several methods were considered to increase the nitroglycerin concentration, including warming the generators and adding four more generators. However, each of these options was reviewed with several explosives experts and were negated for safety reasons. Therefore, it was concluded that we use percutaneous treatment to obtain a dose-response curve.

Nitroglycerin administration on a daily basis either caused an increase in coronary flow during the first part of the 10-day exposure regimen or had no effect during most of the treatment period. However, coronary flow decreased slightly on the last 2 or 3 days of treatment, and the flow appeared to be much less after the end of the 10-day treatment period in those dogs in which we were able to obtain measurements. These observations are the basis for the conclusion that our experimental model is useful in the study of reflex vasoconstriction of coronary arteries after withdrawal from nitroglycerin exposure.

Histopathological examination of tissues taken from implanted animals confirmed that the coronary arteries were not affected by the presence of the Doppler flow probes.

Metabolic studies using ^{13}H-nitroglycerin demonstrated the rapid biodegradation of trinitroglycerin into its dinitro and mononitro components. An interesting observation was the longer biological half-life of the dinitroglycerins after 10 days of inhalation treatment. This is unexpected (no residual nitroglycerin could remain from the previous day's exposure) and would warrant further study if confirmed in continuing studies.

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Appendix

PROBLEMS ENCOUNTERED

Several problems arose during the study. Because this was a developmental study, problems were expected but not specifically anticipated. The major problems were:

- Contamination of the microcircuitry laboratory during assembly of some electronic components delayed production of some of the implantable telemetry equipment.
- The biological life of the Doppler flow probes is about 60 days, instead of longer as had been thought. This caused loss of some animals before we could expose them to nitroglycerin.
- The dogs managed to chew the hard-wired probes with external leads, emphasizing the need for using the totally implantable packages.
- Magnetic switches on the implanted power supplies are not reliable and can be turned on and off by the dog jumping around or getting near magnetic fields undetected. This resulted in run-down batteries.
- Many minor electronics problems developed, most of which were solved but with considerable time and effort.
- Whole-body inhalation exposure of dogs to vapors of nitroglycerin was accomplished, but estimating the doses administered was extremely difficult because, under these conditions, the amount of nitroglycerin breathed by the animals cannot be regulated.

- Analysis for blood levels of nitroglycerin was difficult during the inhalation studies because blood samples cannot be taken during exposure. The electron capture detector on the gas chromatograph could cause some difficulty occasionally because it is a very delicate instrument and was being used heavily.